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Ionic Liquids in Separation Techniques

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1. Introduction

Recently, ionic liquids (ILs) have gained in popularity as unique solvents in different areas of separation techniques. Owing to tunable properties which can be selected by choosing appropriate cationic or anionic constituents, they can be applied in chromatographic and electro-chromatographic methods as mobile phase or stationary phase modifiers or in sample preparations processes as new extraction solvents. Their advantageous properties, from the point of view of separation mechanisms, such as good thermal stability, good extractability for various organic and inorganic ions and the possibility of choosing the optimal viscosity and miscibility with either water or organic solvents make this class of compounds an attractive alternative to classical organic solvents. Furthermore, replacing organic solvents with ILs appears to be much safer for environment regarding the non-flammable and non-volatile nature of these compounds.

This review focuses on applications of ILs in separation techniques: extraction, capillary electrophoresis (CE) and liquid chromatography (LC). So far many studies have been published on the applications of ILs in different separation processes and their number is still growing and attracting great attention. With no doubt, this is connected with huge number of ILs consisting of numerous combinations of various bulky, nonsymmetrical organic cations and inorganic or organic anions. It seems to be obvious that new possibilities of ILs applications will also be discovered in future.

ILs in the form of molten stearates of transition metals were applied firstly in gas chromatography as new liquid stationary phases by Baber et al. (Barber et al., 1959), but since alkylimidazolium-based ILs were introduced for modification of silica capillaries, GC systems have been achieving satisfactory efficiency (Armstrong et al., 1999; Berthod et al., 2001; Anderson & Armstrong, 2003).

In liquid chromatography: either thin layer chromatography (TLC) or high performance liquid chromatography (HPLC), ILs are applied as mobile phase additives as they seem too viscous and non-transparent to UV light to be used as pure solvents. In diluted form, mixed with other low viscosity solvents, they revealed their dual nature responsible for unusual selectivity towards ionic and nonionic analytes. Among the whole group only pyridinium and imidazolium hexafluorophosphates or tetrafluoroborates in reversed-phase chromatographic systems are the most commonly applied for this purpose.

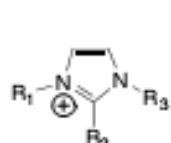
ILs have been used also in capillary electrophoresis (CE) for modification of running buffer and for dynamic coating or covalent attachment to the capillary walls. The main aim of this modification is reverse the electroosmotic flow (EOF) provoked by silanol groups on the inner surface of silica capillaries.

It's worth noting that many reviews devoted to application of ILs in separation techniques systematically appear in the scientific literature (Buszewski & Studzińska, 2008; Marszał & Kaliszan, 2007; Berthod et al., 2008; Sun & Armstrong, 2010; Han & Row, 2010).

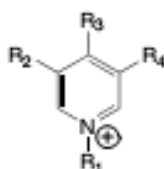
This review focuses on experiments conducted by our group (Flieger, 2006; Flieger, 2009; Flieger & Czajkowska-Żelazko 2011a; Flieger & Czajkowska-Żelazko 2011b; Flieger & Czajkowska-Żelazko 2011c) and described in the literature by others.

2. Physicochemical properties

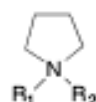
Physicochemical properties and solvation characteristics are useful for choosing appropriate ILs as solvents for extraction or chromatographic methods. Examples for typical cations and anions of ILs used in separation techniques are presented below (sigma-aldrich.com /chemicalsynthesis):



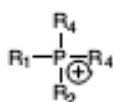
imidazolium



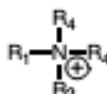
pyridinium



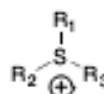
pyrrolidinium



phosphonium



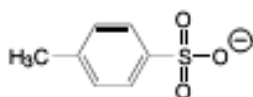
ammonium



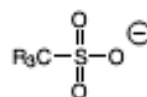
sulfonium



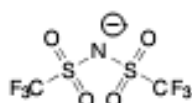
alkylsulfate



tosylate



methanesulfonate

bis(trifluoromethyl-
sulfonyl)imidehexafluoro-
phosphatetetrafluoro-
borate

halide

ILs belong to salt-like materials which are liquid below 100°C and even below room temperature (RTIL-room temperature ionic liquids). The most characteristic properties of ILs cover good thermal stability over the wide range of temperatures 300-400°C, good electric conductivity enabling their application in electro-migration techniques and low vapor pressure; owing to this they are less air-polluting than common organic solvents. The solvent properties of ionic liquids are also interesting. The densities and viscosities of ILs are

generally greater than those of conventional solvents indicating their usefulness in gas chromatography as stationary phase modifiers. On the other hand, they have an extended range of solubility either in polar or non polar solvents, so they could be used in separation techniques including different extraction techniques and as mobile and stationary phase modifiers in liquid chromatography and capillary electrophoresis.

2.1 Solvatochromic parameters

The polarity of ILs can be compared by the use of empirical scales. The most widely applied scale has been based on changes in the charge transfer π - π^* absorption band for the betaine dye, 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl) phenolate also known as Reichardt's dye (Dimroth et al., 1963). The $E_T(30)$ value describing solvent polarity is calculated on the basis of the following equation:

$$E_T(30)(\text{kcalmol}^{-1}) = \frac{28591}{\lambda_{\text{max}}^{\text{abs}}(\text{nm})} \quad (1)$$

Fletcher et al (Fletcher et al., 2001) compared the polarity of BMIM PF₆ with other solvents using $E_T(30)$. Obtained $E_T(30)$ value for Reichardt's betaine dye dissolved in BMIM PF₆ and other solvents reveal that polarity for BMIM PF₆ is similar to that of ethanol.

Poole et al. (Poole & Poole, 2010; Poole, 2004) collected other solvatochromic parameters introduced by Kamlet which have been determined for numerous RTILs. According to this approach solvents could be characterized by their polarity/polarizability (π^*) as well as acidity of hydrogen bond expressed as α value and β value reflecting basicity of the hydrogen-bond. As could be seen in Table 1, presented parameters are clearly related to the structure of ILs. To compare the collected parameters with solvents commonly used in analytical chemistry, methanol, water and acetonitrile have been added at the end of the above-mentioned Table 1.

Wakai and coworkers (Wakai et al., 2005) determined the static dielectric constants of 1-alkyl-3-methylimidazolium ionic liquids by microwave dielectric spectroscopy in the megahertz/gigahertz regime. The obtained results classify the ILs as moderately polar solvents. The observed ϵ -values at 298.15 K fall between 15.2 and 8.8 and decrease with increasing chain length of the alkyl residue of the cation. The anion sequence is trifluoromethylsulfonate > tetrafluoroborate \approx tetrafluorophosphate. The results indicate markedly lower polarities than the ones found by spectroscopy with polarity-sensitive solvatochromic dyes (Wakai et al., 2005).

2.2 Lipophilicity parameters

Lipophilicity of a molecule is measured by its distribution behavior in a biphasic system either liquid-liquid (partition coefficient in 1-octanol-water) or solid-liquid (retention in RP-TLC or RP-HPLC) systems. According to definition suggested by IUPAC lipophilicity expresses the affinity of a molecule for a lipophilic environment. A reference scale representing lipophilicity appears to be the solute distribution between octanol and water. Berthod & Carda-Broch (Berthod & Carda-Broch) proposed another lipophilicity scale measuring ionic liquid: BMIM PF₆-water distribution constants. Relationship between the obtained values and respective octanol-water coefficients for a series of aromatic compounds differing in acid-base properties revealed that only the neutral compounds or ionizable ones with zwitterionic properties showed similar distribution behavior in the

Ionic liquid	Reichardt's dye		Kamlet-Taft		
	E _T (30)	E _T ^N	π *	α	β
Ethylammonium nitrate	61.6	0.95	1.24	0.85	0.46
n -Propylammonium nitrate	60.6	0.92	1.17	0.88	0.52
Di-n -propylammonium thiocyanate	63.3	1.01	1.16	0.97	0.39
1-Butyl-3-methylimidazolium Hexafluorophosphate	52.3	0.67	1.03	0.63	0.21
1-Butyl-3-methylimidazolium Tetrafluoroborate	52.5	0.67	1.05	0.63	0.38
1-Butyl-3-methylimidazolium Trifluoroacetate	51.1	0.63			
1-Butyl-3-methylimidazolium Trifluoromethanesulfonate	52.3	0.67	1.01	0.63	0.46
1-Butyl-3-methylimidazolium Bis(trifluoromethylsulfonyl)imide	51.5	0.64	0.98	0.62	0.24
1-Butyl-2,3-dimethylimidazolium Bis(trifluoromethylsulfonyl)imide	49.3	0.57	0.99	0.45	0.26
1-Butyl-2,3-dimethylimidazolium Tetrafluoroborate	49.4	0.58	1.08	0.40	0.36
1-Ethyl-3-methylimidazolium Bis(trifluoromethylsulfonyl)imide	52.6	0.68			
1-Ethyl-3-methylimidazolium Tetrafluoroborate	53.7	0.71			
1-Hexyl-3-methylimidazolium Bis(trifluoromethylsulfonyl)imide	51.9	0.65	0.98	0.65	0.25
1-Hexyl-3-methylimidazolium Tetrafluoroborate	53.6	0.71			
1-Hexyl-3-methylimidazolium Trifluoromethanesulfonate	52.5	0.67	0.98	0.67	0.52
1-Octyl-3-methylimidazolium Bis(trifluoromethylsulfonyl)imide	51.0	0.63	0.97	0.60	0.28
1-Octyl-3-methylimidazolium Tetrafluoroborate	52.4	0.67			
1-Octyl-3-methylimidazolium Hexafluorophosphate	50.0	0.60	0.88		
Water	63.1	1.00	1.09	1.17	0.18
Methanol	55.8	0.77	0.60	0.93	0.62
Acetonitrile	51.9	0.65	0.80	0.35	0.38

Table 1. Solvent strength determined by solvatochromism for representative room temperature ionic liquids and traditional organic solvents (Poole & Poole, 2010; Poole, 2004). compared systems. As can be seen in Fig.1, the amine-containing compounds showed higher affinity to ionic liquid whereas acidic compounds to 1-octanol phase. This result is in consistent with the solvation model proposed by Abraham et al. (Abraham et al., 2003) indicating that BMIM PF₆ has a greater affinity for electron lone pair interactions. More data for liquid-ionic liquid partition coefficients of different organic compounds were described for the biphasic systems containing alkyl ammonium ionic liquids: ethylammonium nitrate, n-propylammonium nitrate, or di-n -propylammonium thiocyanate and hexane, toluene, octan-1-ol, or dichloromethane (Poole, 2007; Shetty et al., 1987; Shetty et al., 1990) or aqua biphasic systems formed by 1-butylammonium-3-methylimidazolium hexafluorophosphate and water or heptane (Berthod & Carda-Broch, 2003; Khachatryn et al., 2005; Carda-Broch et al., 2003) or the 1-octyl-3-methylimidazolium hexafluorophosphate-water biphasic system (Liu et al., 2004).

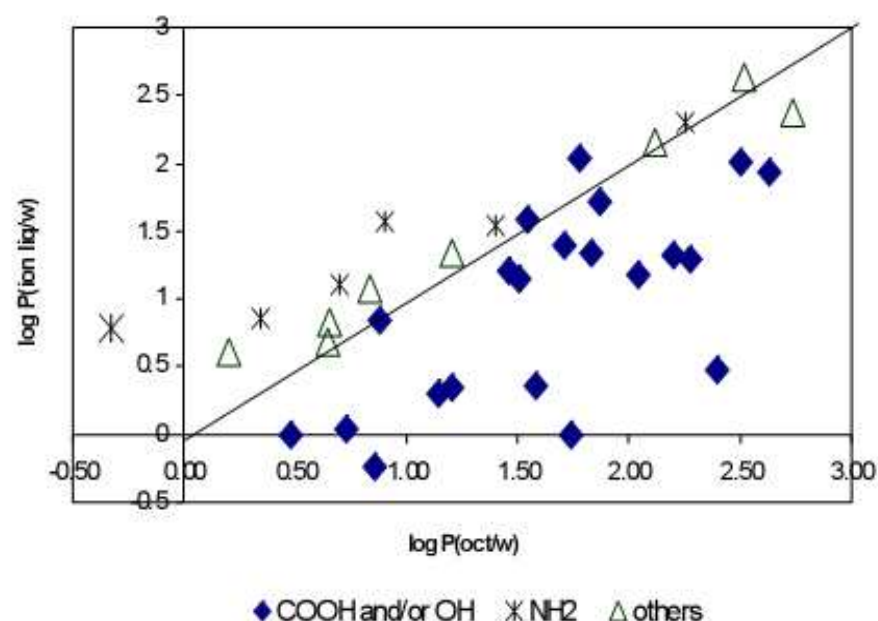


Fig. 1. Ionic liquid/water distribution coefficients compared to the octanol/water values (log scale). Crosses: amino-aromatic compounds, open triangles: neutral compounds or compounds with both acidic and basic substituents, filled-diamond: acidic and/or phenolic compounds (<http://www.mariecurie.org/annals/volume3/berthod.pdf>).

IL additives have been also applied as a mobile phase modifiers in RP-HPLC systems used for determination of the chromatographic lipophilicity parameters $\log k_w$ of ionized forms of basic drugs (Marszałł & Kaliszan, 2007). The correlation of $\log k_w$ versus the reference lipophilicity parameters $\log P_{o/w}$ was evidently better when ionic liquid EMIM BF_4 was added to the mobile phase at very small concentration (1.5%).

To determine the lipophilicity of ionic liquids, different stationary phases have been tested such as Supelcosil LC-8-DB, Symmetry C8, ACE 5 C18, Symmetry C18 with aqueous acetonitrile buffered at pH 3.55 mobile phases (Molikova et al., 2010a). In this study the $\log k_w$ parameters were correlated with $\log P$ calculated by the HyperChem software. Good correlations were found for homologous alkylimidazolium cations analyzed by RP HPLC especially in the case of the Symmetry C8 column ($R^2 = 0.9966$).

3. Liquid-liquid extraction systems

3.1 Aqueous biphasic systems based on ionic liquid

It has been recently demonstrated that hydrophilic ionic liquids induce formation of aqueous biphasic systems (ABS) or aqueous two-phase systems (ATPS) in the presence of inorganic salts with water-structuring properties (Gutowski et al., 2003; Shehong et al., 2005). According to Flory (Flory, 1953) the ATPS in classical mixtures consisting of two aqueous solution of polymers or a polymer and salt, are formed when enthalpy of interaction between the polymer molecules is bigger than the loss in entropy. It appears that the addition of cosmotropic salt to an aqueous solution of a hydrophilic ionic liquid also causes a salting out effect leading to liquid-liquid demixing and finally to the formation of two water-rich immiscible phases where upper phase contains ionic liquid and lower one concentrated salt. Abraham et al. proved that ATPS could be regarded as novel liquid

partitioning systems. (Abraham et al., 2003). Considering that, stability, activity and enantioselectivity of enzymes have all been enhanced in aqueous solution of ionic liquids (Dreyer & Kragl, 2008; Dreyer and Kragl, 2009) and water-rich phases protect biomolecules against denaturation. ATPS have been used mainly in biotechnology for the separation of biomolecules, such as cells, organelles, membrane fractions and proteins (Walter & Johansson, 1994; Albertsson, 1986; Rito-Palomares, 2004; Gunduz & Tolga, 2004; Roobol-Boza et al., 2004; Agasøster, 1998). This method appears to be an attractive alternative to conventional extraction methods such as liquid-liquid extraction and solid-phase extraction, to simultaneously carry out isolation, purification and enrichment of biomolecules (Rito-Palomares, 2004; Gunduz & Tolga, 2004; Roobol-Boza et al., 2004). Apart from proteins like bovine serum albumin, lysozyme, trypsin, myoglobin (Pei et al., 2009; Du et al., 2007; Dreyer & Kragl, 2008; Dreyer and Kragl, 2009) ATPS have been used for the recovery of small organic and inorganic molecules such as metal ions, radiochemicals, dyes, drug molecules (Huddleston et al., 1999; Willauer et al., 2002; Zhu et al., 2001; Bridges & Rogers, 2008). Short chain alcohols (Gutowski et al., 2003), phenol (Chen et al., 2009), testosterone and epitestosterone (He et al., 2005), penicillin G (Liu et al., 2006), opium alkaloids (Li et al., 2005a), L-tryptophan (Neves et al., 2009; Ventura et al., 2009; Louros et al., 2010), antibiotics (Dominguez-Perez et al., 2010), caffeine and nicotine (Freire et al., 2010) have been analysed as partitioning solutes so far.

Biphasic liquid systems could find special applications in countercurrent chromatography (CCC) where both the mobile and stationary phase are immiscible liquids. Berthod and coworkers used biphasic liquid system consisting of water-acetonitrile-BMIM PF₆ for determination of the distribution constants of different organic compounds (Berthod & Carda-Broch, 2004).

3.1.1 ATPS driving forces

Most water-soluble room temperature ionic liquids could be thought of as chaotropic salts. That is why after addition of water-structuring salts (kosmotropic salts) they are salted out and aqueous biphasic systems are formed (Bridges et al., 2007; Zafarani-Moattar & Hamzehzadeh, 2009; Neves et al., 2009; Deng et al., 2007; Pei et al., 2007). Classification of salts is based on the Hofmeister series ordered from kosmotropic to chaotropic accordingly to the ability of salt to protein precipitation. Alternatively to ionic reagents appropriate sugars such as fructose and sucrose could be used as kosmotropic agents known also as water-structuring additives. The Hofmeister series was established in 1888 (Hofmeister, 1888).

Kosmotropic ions	Chaotropic ions
citrate ³⁻ > sulfate ²⁻ > phosphate ²⁻ > F ⁻ > Cl ⁻ > Br ⁻ > I ⁻ > NO ₃ ⁻ > ClO ₄ ⁻	
Strongly hydrated	weakly hydrated
N(CH ₃) ₄ ⁺ > NH ₄ ⁺ > Cs ⁺ > Rb ⁺ > K ⁺ > Na ⁺ > H ⁺ > Ca ²⁺ > Mg ²⁺ > Al ³⁺	
weakly hydrated	strongly hydrated

Originally it was stated that the influence of ions on macromolecules properties was connected with structuring or breaking of bulk water structure. However recent studies have demonstrated that either direct ion-macromolecule interactions or interactions with water molecules in the first hydration shell of the macromolecules govern the Hofmeister effect as well as phase separation in ATP systems (Zhang & Cremer, 2006). Thus, in ATPS

systems based on ionic liquid the competition between the ionic liquid and inorganic salt for water molecules appears to be the main driving force responsible for phase separation. It can be confirmed by the thermodynamic approach utilizing the Gibbs free energy of hydration ΔG_{hyd} . Kosmotropic ions with a large negative ΔG_{hyd} possess the higher affinity for water; in this way forcing the flow of water far away from the ionic liquid decreasing its solubility (Bridges et al., 2007). Typically the following inorganic salts have been used as kosmotropes: ammonium, potassium and sodium of phosphates, sulfates, carbonates or citrates whereas ionic liquids chosen for ATPS formation are mainly hydrophilic 1,3-dialkylimidazolium-based water soluble salts.

To characterize the obtained biphasic systems, the phase diagrams determined by the cloud-point titration are usually constructed. The binodal has been described by an empirical model developed by Merchuk and coworkers (Merchuk et al., 1998). The mathematical representation of the curves fitted the best to experimental data was the following:

$$Y = M_1 \exp[(M_2 X^{0.5}) + (M_3 X^3)] \quad (2)$$

Bridges and coworkers (Bridges et al., 2007) studied the BMIM Cl- K_3PO_4 phase diagram. As can be seen in Fig.2 the binodal separates the monophasic region from the two phase region which is located above the binodal. The tie line length measures the relative divergence in two phases. According to Merchuk (Merchuk et al., 1998) the phase ratio can be derived from the inverse lever rule. Composition of biphasic systems at any point of tie line could be determined by the intersecting points of appropriate tie line with the binodal curve.

Some papers described (Li et al., 2005a; Shehong et al., 2005) different salts which were also tested for the formation of ATPS with BMIM Cl (Fig.3). It appeared that ATPS can be formed only by adding appropriate amount of alkali or alkaline salts, such as KOH, NaOH, K_3PO_4 , K_2HPO_4 , K_2CO_3 or Na_2HPO_4 whereas acidic or neutral salts, such as KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$, NaCl or KCl did not create biphasic system. The abilities of the salts for phase separation can be ordered as follows:

$$\text{K}_2\text{CO}_3 \approx \text{K}_3\text{PO}_4 \approx \text{K}_2\text{HPO}_4 > \text{KOH}$$

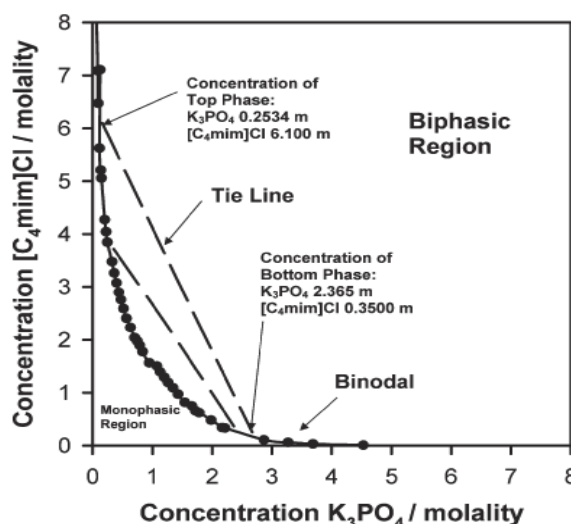


Fig. 2. The phase diagram for [C₄ mim]Cl- K_3PO_4 illustrating the binodal (—) and the tie lines (---). (Bridges et al., 2007).

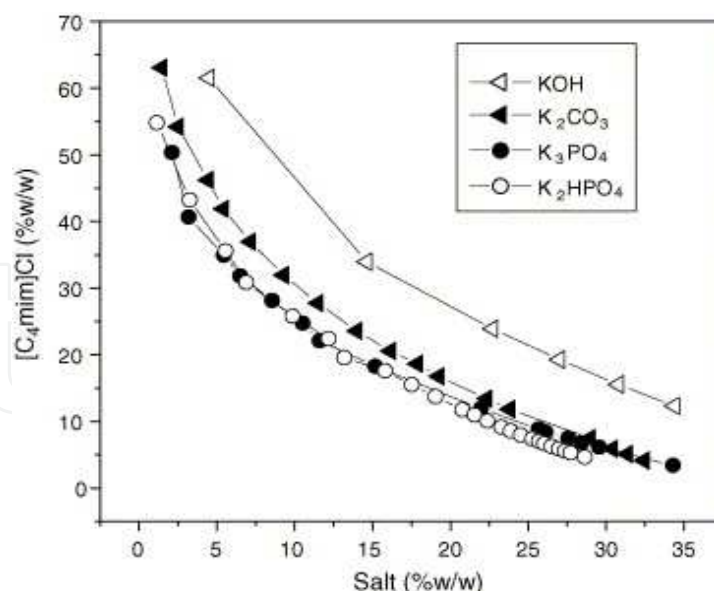


Fig. 3. Phase diagrams for the $[C_4mim]Cl$ /salt/water systems at 22 °C. In the region above the binodial curve, the system is separated into two phases, below the binodial curve; the system is a homogeneous phase (Shehong et al., 2005).

Several other papers have been also dealing with phase diagrams of IL-based ABS (Pei et al., 2007; Zafarani-Moattar & Hamzehzadeh, 2007; Bridges et al., 2007; Visak et al., 2007; Najdanovic-Visak et al., 2007; Wu et al., 2008a,b,c; Zhang et al., 2007,2008). Research regarding the use of IL-based ATPS has been mostly focused on the influence of inorganic salts using first of all imidazolium-based ILs. Bridges et al (Bridges et al., 2007) determined the ability of different ionic liquids chlorides to ATPS formation using the strongest kosmotropic salt K_3PO_4 (Fig.4). An overall chaotropic order of the studied salts can be arranged as follows: $P_{4444}Cl > N_{4444}Cl \gg [C_4py]Cl \gg [C_4mmim]Cl \approx [C_4mim]Cl$. This order reflects the increased chaotropicity of ionic liquids due to the differences in the cation structure. Fig.4 shows small, but still visible, quantitative differences in the binodal curves for the phosphonium-based ILs. The ability of the ILs for phase separation can be described by the following order: $[P_{4444}]Br > [P_{i(444)4}][Tos] > [P_{4441}][MeSO_4]$.

In turn Louros (Louros et al., 2010) studied phosphonium-based ILs which are more effective in promoting ATPS in comparison to the imidazolium-based IL with similar anions (Fig.5).

3.1.2 The recovery of proteins and amino acids

The partitioning behavior of proteins in IL-based ATPS is not clear. Some authors state that the salting-out effect and the electrostatic interactions are responsible for protein extraction into the IL-containing phase (Dreyer & Kragl, 2008). This model is based on published data illustrating the visible influence of the surface charge of the proteins and their molecular weight on the partitioning in ATPS systems. It appears that only bigger proteins with the negative charges on the surface possess higher affinity to upper IL-rich phase. Interactions between negatively charged amino acid residues and the cation of the IL were identified as the main driving forces for the extractions. In contrast to the above idea, another study elaborated on by Pei (Pei et al., 2009) points out hydrophobic interactions as a dominating factor in extraction process. The authors based their conclusion on thermodynamic calculations indicating that, the extraction is controlled by entropy changes, similarly to hydrophobic interactions. Determined TAS values were always greater than the ΔH at each

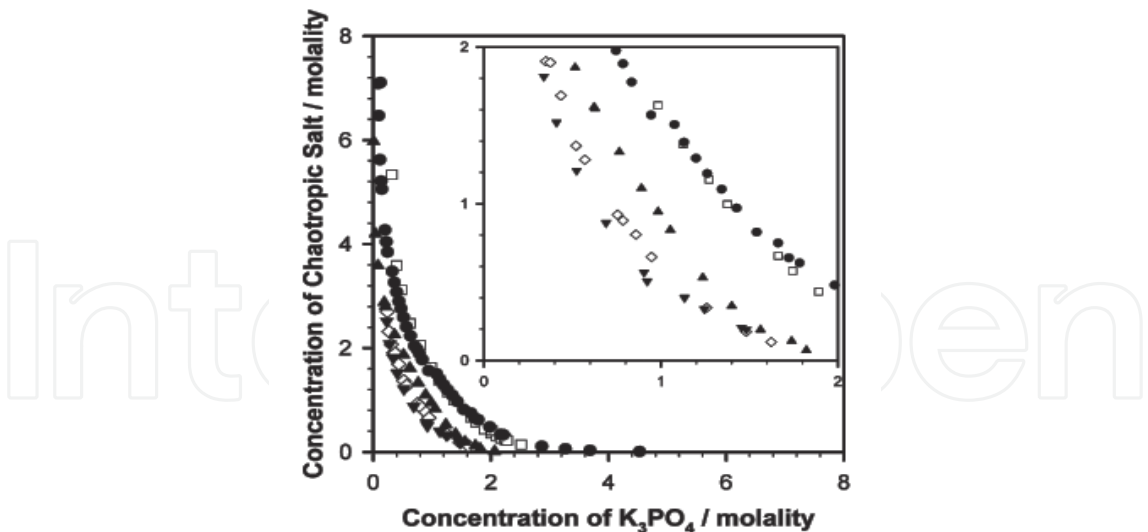


Fig. 4. Phase diagrams of K_3PO_4 , with (●) $[C_4mim]Cl$, (□) $[C_4mmim]Cl$, (▲) $[C_4py]Cl$, (◇) $N_{4444}Cl$, and (▼) $P_{4444}Cl$. (Bridges et al., 2007).

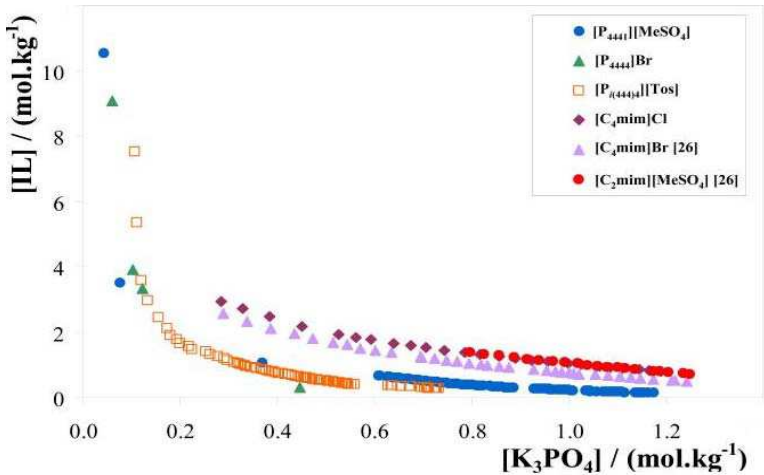


Fig. 5. Phase diagrams for the phosphonium-based ILs and $[C_4mim]Cl$ ternary systems composed by IL + K_3PO_4 + H_2O at 298 K. (Louros et al., 2010).

case of ATPS systems containing ionic liquids differing in hydrophobicity of cation: $[C_4mim]Br$, $[C_6mim]Br$, $[C_8mim]Br$.

A series of dinitrophenylated amino-acids was studied in biphasic systems based on 1-decyl-3-methylimidazolium tetrafluoroborate and 1-hexyl-3-methylimidazolium tetrafluoroborate at slightly basic conditions fixed at 7.4 using sodium phosphate buffer (Rodriguez et al., 2007). Authors observed that amino-acids distribute preferentially to the ionic liquid phase according to the length of their chain.

3.1.3 The recovery of antibiotics, hormones and alkaloids

IL-based ATPS systems have been used for recovery of antibiotics especially the ones produced in fed-batch fermentation process by microorganisms. Lui (Lui et al., 2005, Lui et al., 2006) elaborated on methods of penicillin G extraction from fermentation media with an ATPS based on BMIM BF_4 and BMIM Cl and NaH_2PO_4 as an inorganic salt. Improvement of the yield of extraction has been achieved with increasing phosphate concentration in the lower

phase and increasing ionic liquid concentration in the upper phase. The maximum extraction yield of above 90% has been reached with 40% kosmotropic salt and 20% of BMIM Cl. In turn Yangyang and coworkers (Yangyang et al., 2009) used BMIM PF₆ simply to support antibiotic extraction by conventional ATPS system containing an imidazolium-terminated polyethylene glycol allowing efficient (96%) extraction of penicillin G. A second biphasic system based on IL and adjusted to slightly basic pH can recover I-PEG which is further extracted into slightly acidic water phase at third step. This method enables recycling not only of the imidazolium-terminated polymer but also of the added ionic liquid.

Despite of the great extractive potential of IL-based ATPS systems for fundamental biomolecules, most studies have been dealing with phase diagrams. Since the ionic liquid containing phase is compatible with liquid chromatography and UV detection, IL-based ATPS appear to be suitable for real samples investigation. As partitioning solutes only a few alkaloids have been analyzed so far (Freire et al., 2010; Li et al., 2005). Lui and coworkers (Lui et al., 2005) demonstrated aqueous two-phase systems (ATPS) based on an ionic liquid, 1-butyl-3-methylimidazolium chloride BMIM Cl/K₂HPO₄ in combination with high-performance liquid chromatography (HPLC) for analysis of the major opium alkaloids: codeine and papaverine in *Pericarpium papaveris* (Fig.6). The recoveries of codeine and papaverine were 90.0–100.2% and 99.3–102.0%, respectively. More recently Freire (Freire et al., 2010) reported strong ability of a series of IL-based ATPS for extraction of caffeine and nicotine in a single-step procedure. The authors focused on testing the concentration of inorganic salts and the usefulness of several ionic liquids differing in the anion and the cation in the aqueous phase and more complex matrixes such as human urine. The results showed that the extraction of both alkaloids significantly improved in human urine samples in comparison to simpler aqueous phases. Authors concluded that the presence of a biological matrix containing additionally neutral salt NaCl and urea with chaotropic properties favored the alkaloid partitioning for the IL phase.

Li studied BMIM Cl-K₂HPO₄ (Li et al., 2005) ATPS system for extraction of two alkaloids - morphine and papaverine used as abused drugs. The maximum extraction efficiency which was about 96% for papaverine and 65% for morphine was obtained for a system consisting of 3% IL and 40% of K₂HPO₄ within the temperature range from 15 to 65°C.

Because the ion exchange between salt and IL can complicate separation procedure, the use of sugars (a, b, c Wu et al. 2008) and aminoacids (Zhang et al., 2007; Dominiguez-Perez et al., 2010) has been suggested to form IL-based ATPS. Perez evaluated the capability of imidazolium-based ILs (BMIM) differing in the type of anion (triflate, dicyanamide, tetrafluoroborate) and amino acids proline and lysine to promote ATPS. The suitability of the proposed ATPS was further checked according to their potential for the extraction of caffeine and antibiotic ciprofloxacin in two forms differing in solubility in water. On the basis of ternary phase diagrams for the examined systems ability of the aminoacids to form ATPS could be expressed as the distance between the origin and the binodal curves. It appeared that more soluble lysine leads to a more pronounced salting-out effect. In turn the hydrogen-bond basicity of the anion composing the IL ([BF₄]⁻ > [CF₃ SO₃]⁻ > [N(CN)₂]⁻) decides about the ability of [C₄ mim]-based ILs for aqueous phase separation.

Aqueous two-phase systems (ATPS) based on hydrophilic ionic liquid 1-butyl-3-methylimidazolium chloride and K₂HPO₄, coupled with RP-HPLC were proposed for analysis of testosterone (T) and epitestosterone (ET) in human urine (He et al., 2005). The method has been reached satisfactory extraction efficiencies 80–90% and detection limits of 1 ng/mL and linear ranges of 10–500 ng/mL for both compounds (Fig.7).

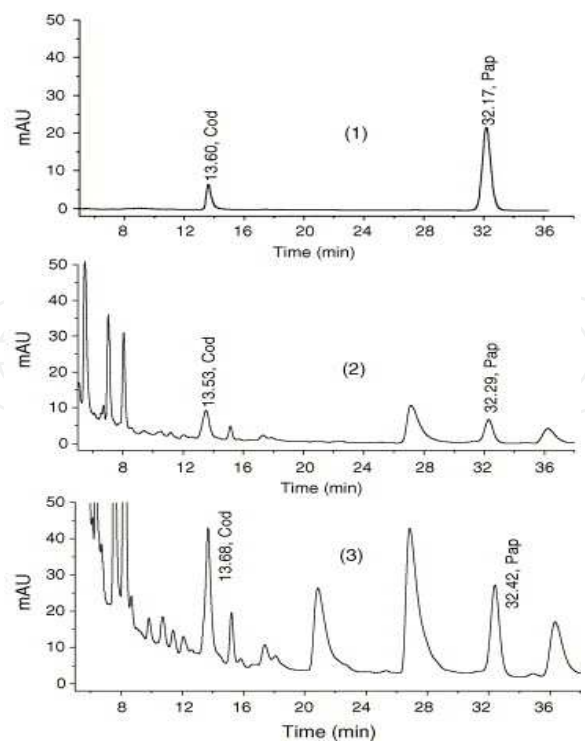


Fig. 6. Chromatogram of (1) standard solution of 10 µg/ml codeine (Cod) and 4 µg/ml papaverine (Pap); (2) methanol LLE of Pericarpium papaveris sample; (3) ATPS extraction of Pericarpium papaveris sample (Li et al., 2005).

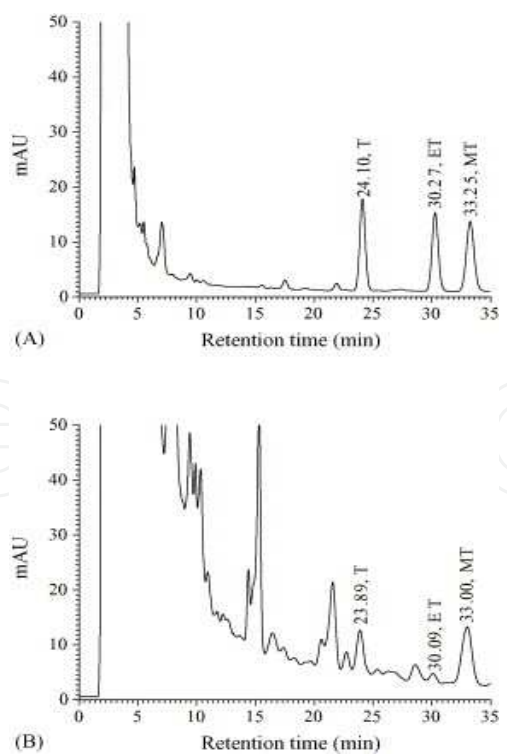


Fig. 7. HPLC chromatograms with UV detection of: (A) SFUS spiked with standard (300.0 ng/mL) and I.S. (333.3 ng/mL); (B) a human urine sample added with I.S. (333.3 ng/mL). Column: Zorbax SB-C₁₈. Mobile phase: 39/61% (v/v), acetonitrile-water. Flow rate: 1.0 mL/min. UV detection at 245 nm. (He et al., 2005).

3.2 Liquid-liquid extraction by hydrophobic ionic liquids

Pure ILs with hydrophobic properties can replace organic solvents in liquid-liquid extraction. Examples of different extraction systems are presented in Table 2. It should be emphasised that the extractability of acids (Matsumoto et al., 2004) and amino acids (Smirnova et al., 2004) could be enhanced by the addition of different extractants such as tri-*n*-butylphosphate or crown ether respectively to the ionic liquid phase. If analytes exist in ionic form, the extraction occurs by an ion-exchange mechanism involving transfer of appropriate ions to maintain electroneutrality. In this case, stronger hydrogen-bonding interactions will generate tetrafluoroborates owing to the stronger effective charge in comparison to hexafluorophosphates (Fan et al., 2008), taking into account extraction of phenols. In turn increasing the hydrophobicity of the cation provided decreases the extraction efficiency. Ionic liquids are also good extractants of neutral compounds (benzene derivatives) from water (McFarlane et al., 2005). The partition coefficients obtained in IL-water system correlate with that ones determined by classical shake-flask method in *n*-octanol-water extraction. Addition of *n*-octanol to ionic liquid phase can significantly improve extraction of carboxylic acids. Besides imidazolium ionic liquids also ionic liquids with quaternary ammonium cations, such as tetrahexylammonium dihexylsulfosuccinate and trioctylmethylammonium salicylate could be successfully applied to extraction of neutral compounds. Egorov (Egorov et al., 2008) showed that the novel RTILs are a more efficient solvent for extraction of phenols and amines than commonly applied for this purpose imidazolium based ILs: HMIImTf₂N and DMImTf₂N.

Compounds extracted into ionic liquid phase can be recovered by back extraction enabling phase-forming compounds recycling. Such multi-steps extraction procedures have been applied in biotechnology using the ILs in large-scale processes.

Hydrophobic ILs have been considered as a solvent for biocatalytic reactions. It was shown that the reaction of 2-octanone reduction to 2-octanol catalysed by alcohol dehydrogenase is faster when IL BMIM (CF₃SO₂)₂N is present in comparison to organic solvent applied so far (Eckstein et al., 2004). Additional beneficial effects of ILs is reduction of the toxicity towards the cell and possibility of their application in whole-cell biocatalysis (Weuster-Botz, 2007; Pfruender et al., 2004). This advantageous effect comes from excellent solvent properties of ILs extracting either products or reaction substrates.

RTILs are being considered for selective extraction either in macro or micro scale. The isolation of target compounds from chemical process systems for instance alcohols from fermentation liquors (Chapeaux et al., 2008; Swatloski et al., 2002; Najdanovic-Visak et al., 2002), aromatic compounds from petroleum products (Arce et al., 2007a,b; Arce et al., 2009; Arce et al., 2008), aromatic sulfur-containing compounds (Mochizuki & Sugawara, 2008; Alonso et al., 2007; Eßer et al., 2004) from diesel-fuel represent the use of ILs in large-scale processes.

In turn liquid-phase microextraction techniques such as dispersive, single-drop, hollow-fiber based liquid-phase microextractions and solid-phase with liquid film represent miniaturized systems also utilizing ionic liquids as the extraction solvents. The low solubility, the low vapor pressure, high viscosity of ILs allow the use of long extraction time and eliminate evaporation losses. Using 1-hexyl-3-methylimidazolium tetrafluoroborate in temperature-controlled dispersive liquid-phase microextraction pyrethroid pesticides (Zhou et al., 2008) were isolated from aqueous solution. Headspace liquid-phase microextraction using 1-octyl-3-methylimidazolium hexafluorophosphate was applied for extraction of halomethanes, alkyl aromatic compounds (Aguilera-Herrador et al. 2008a,b, 2009), and

chlorophenols (Peng et al., 2007). 1-Butyl-3-methylimidazolium hexafluorophosphate enabled the separation of chlorobenzenes and chloroanilines (Vidal et al., 2007). Amphetamine and methamphetamine were extracted from urine by the use of solid-phase microextraction employing an immobilized liquid film of 1-ethoxyethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide on a silicone matrix (He et al., 2009).

4. Determination of ionic liquids

Taking into account widespread application of ionic liquids in the chemical industry, they should also be considered as analytes determined in different matrices, solid or liquids coming from environment. Several techniques such as capillary electrophoresis (Qin et al., 2002; Markuszewski et al., 2004), isotachopheresis (Kosobucki & Buszewski, 2008), high performance liquid chromatography (Stepnowski et al., 2003; Ruiz-Angel & Berthod, 2006; Buszewski et al., 2006; Kowalska & Buszewski, 2006), ion chromatography (Stuff, 1991; Isildak & Covington, 1993; Markowska & Stepnowski, 2008; Molikowa et al., 2010) (Fig.8) have been applied for determination of ILs.

Cations and anions derived from ionic liquids can be analyzed separately or simultaneously. In the first case, two different analytical methods should be applied. Separation of ILs cations can be analyzed on a cation exchange column (Stuff et al., 1991; Molikowa et al., 2010) whereas halides anion by the use of anion exchanger also by ion chromatography (Hao et al., 2008).

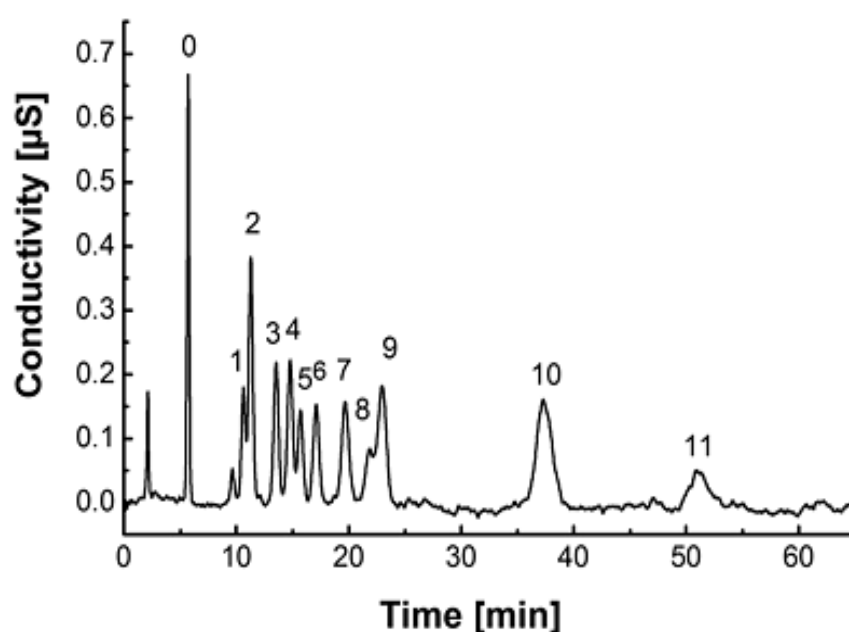


Fig. 8. Chromatogram of separation of ionic liquid cations mixture with isocratic elution for mobile phase consisting of 30% v/v of acetonitrile and: 70% v/v of 5mM MSA (methanesulfonic acid). Key:0-unknown compound,1-EEIM,2-PMIM,3-BMIM,4-BEIM,5-MBPY,6-AMIM,7-BzMIM,8-EBzMIM,9-HMPY,10-HMIM,11-OMIM. The cations of IL were analyzed on a Dionex IonPac CS15 column (250mm x 8.5mm) (Molikowa et al., 2010).

Extracted compounds	Ionic liquid	environment	Ref.
acetic, glycolic, propionic, lactic, butyric, pyruvic	The hexafluorophosphates of 1-butyl-, 1-hexyl-, 1-octyl-3-methylimidazolium containing tris-n-butylphosphate	water	Matsumoto et al., 2004
phenols	1-butyl-3-methylimidazolium hexafluorophosphate	Aqueous solution adjusted to $pH < pK_a$	Khachatryan et al., 2005
phenol, tyrosol, p-hydroxybenzoic acid	1-alkyl-3-methylimidazolium hexafluorophosphates and tetrafluoroborates	Aqueous solution	Vidal et al., 2004
phenol, bisphenol A, pentachlorophenol, 4-octylphenol, 4-nonylphenol	1-methyl-3-alkyl imidazolium hexafluorophosphate 1-methyl-3-alkylimidazolium tetrafluoroborate	Aqueous solution $pH < 7$	Fan et al., 2008
amino acids: arginine, glycine, tryptophan, leucine, alanine, lysine, valine	1-butyl-3-methylimidazolium hexafluorophosphate containing the crown ether dicyclohexano-18-crown-6	Aqueous solution at $pH\ 1.5-4.0$	Smirnova et al., 2004
amino acids (valine, leucine, tyrosine, phenylalanine, and tryptophan)	1-butyl-3-methylimidazolium hexafluorophosphate, 1-hexyl-3-methylimidazolium hexafluorophosphate and tetrafluoroborate, and 1-octyl-3-methylimidazolium tetrafluoroborate	Dependence on pH of aqueous solution	Wang et al.,
amoxicillin and ampicillin	1-octyl-3-methylimidazolium tetrafluoroborate	aqueous solution $pH = 8$	Soto et al., 2005
azo dyes (naphthalene sulfonic acids)	N-butyl-N-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide	aqueous solution	Vijayaraghavan et al., 2006
toluene, cyclohexanone, nonan-1-ol, acetic acid and hexanoic acid	(1-alkyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, hexafluorophosphate, trihexyl-tetradecyl phosphonium bis(trifluoromethylsulfonyl) imide, dodecylbenzene sulfonate, trihexyl-tetradecyl phosphonium methanesulfonate	water at various pH , temperature, sodium chloride concentration	McFarlane et al., 2005
phenols and amines	tetrahexyl ammonium dihexylsulfosuccinate, trioctylmethyl ammonium salicylate	aqueous solution	Egorov et al., 2008.
organic compounds	di-n-propylammonium thiocyanate	hexane	Shetty et al., 1990

Table 2. Examples of different liquid-liquid extraction systems based on ILs.

However, the most common method of ILs cations determination appears to be high performance liquid chromatography in reversed-phase mode (Ruiz-Angel & Berthod, 2006; Ruiz-Angel & Berthod, 2008; Stepnowski et al., 2003). Using C-18 bonded phase with organic-aqueous mobile phase, selectivity of separation especially for more polar cations is rather poor. Distorted and even split peaks may appear in the chromatogram depending on the nature and concentration of the injected ILs (Ruiz-Angel & Berthod, 2008). Significant improvement of peak shape and reproducibility of retention factors could be achieved by addition of appropriate acidic buffer, small amount of acid or significant amounts of a salt containing a chaotropic anion to the mobile phase. To separate the mixture of ionic liquids differing with the hydrophobicity of cations, gradient separation is required. Stepnowski and coworkers proposed very efficient chromatographic system with electrospray ionization mass detection for separation of selected 1-alkyl and 1-aryl-3-methylimidazolium-based RTIL cations (Stepnowski et al., 2003). The results obtained on C₈ MetaSil Basic column (Fig.8) were more efficient than that ones performed on C18 column and organic aqueous mobile phase with addition of different salts proposed by Ruiz-Angel and Berthod (Ruiz-Angel & Berthod, 2006). The key matter in determination of ionic liquids appears to be simultaneous analysis of the cations and anions of ILs. This approach involves the use of a mixed bed cation and anion exchange material or cation and anion exchange columns connected in tandem. Markowska & Stepnowski proposed the tandem of columns consisting of silica-based strong anion exchange packing (Phenosphere SAX with quaternary ammonium groups) and strong cation exchange packing (Luna SCX, with sulfonic groups) for simultaneous separation of ionic liquid components in one run followed by conductometric detection (Markowska & Stepnowski, 2008). Separation of a mixture of ionic liquids is presented in Fig.10. The limits of detection (LOD) values obtained by the use of this method were in the range from 0.008 to 0.050 mM; which were similar to the minimum detectable levels determined in the case of capillary electrophoresis ranging from 0.2 to 1.9 mg/L (Soga & Ross, 1999).

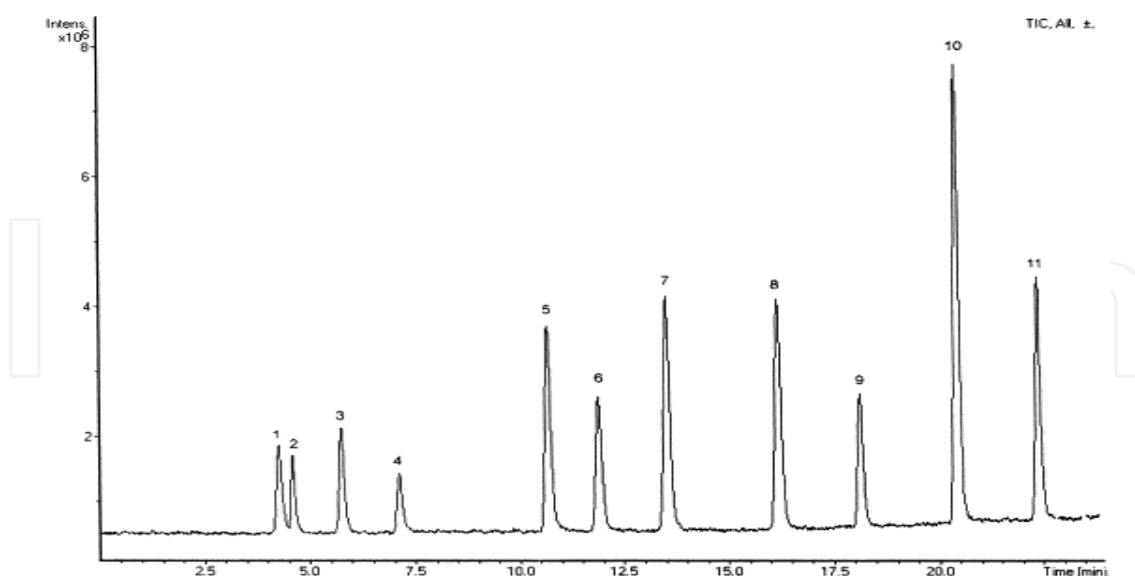


Fig. 9. Gradient separation of the test mixture. Column RP C₈ MetaSil Basic 250×4.6 mm I.D. 5 μm (Varian). Mobile phase A, acetonitrile; B, water (1% acetic acid/20 mM ammonium acetate) 0 min. 10% B, 10 min. 20% B, 25 min. 50% B. 1-EEIM Br, 2- PMIM Br, 3- PEIM Br, 4- BMIM Cl, 5- BzMIM BF₄, 6-AMIM Cl, 7- EBzMIM Cl, 8- pMBzMIM Cl, 9-HMIM Cl, 10- HEIM Cl, 11- HpMIM Cl. (Stepnowski et al., 2003).

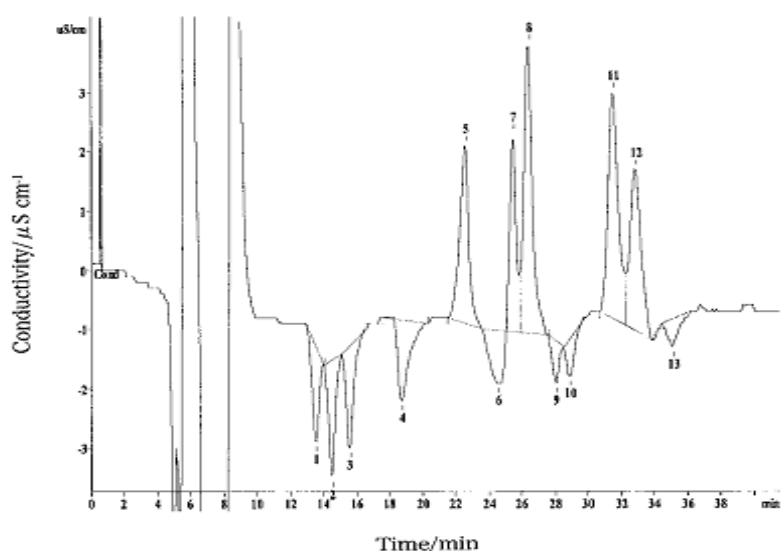


Fig. 10. Separation of a mixture of ionic liquid cations and anions (concentration 0.5 mM each) with use of tandem ion exchange columns. 1, DMIM; 2, NMIM; 3, OMIM; 4, HMIM; 5, Tf_2N^- ; 6, AMIM; 7, PF_6^- ; 8, BF_4^- ; 11, Br^- ; 12, Cl^- ; 13, BMIM; signals 9,10 unidentified. Conductivity detection. Mobile phase: 5 mM potassium hydrogen phthalate/15 mM phthalic acid, 45% (v/v) acetonitrile pH 3.05. Absolute conductivity of mobile phase, 476 $\mu\text{S}/\text{cm}$; flow rate, 1 mL/min; injection volume, 100 μL . Columns in the order: strong anion exchange Phenosphere SAX, strong cation exchange Luna SCX. (Markowska & Stepnowski, 2008).

5. Application of ionic liquids in Liquid chromatography

5.1 Mobile phase modification with ILs

The application of ionic liquids in liquid chromatography has been restricted to mobile phase modification at low percent levels. This limitation is connected with, first of all, high viscosities which are higher than typical chromatographic solvents and secondly with low UV transparency. As mobile phase additives ILs could be advantageous, coating residual hydroxyl groups on the silica surface, modifying the stationary phase or acting as ion-pairing agents. Owing to these additives improvement of peak symmetry, efficiency and separation selectivity could be achieved. Suppression of deleterious silanol effects as a result of incomplete silanization of silica based packings have been described and compared to typical suppressor such as alkylamines used in the past for this purpose (Kaliszan et al., 2004; Marszałł et al., 2006a; Marszałł et al., 2005; Ruiz-Angel et al., 2006). It was found that ionic liquids of the imidazolium tetrafluoroborate class, added to mobile phases at concentrations of 0.5–1.5% (v/v), blocked free silanols exceeding the standard mobile phase additives, like triethylamine, dimethyloctylamine and ammonia either in thin-layer chromatographic separations or high-performance liquid chromatography of strongly basic drugs. To evaluate the silanol-suppressing potency of ILs the dual retention model based on Nahum and Horvath equation has been applied (Marszałł et al., 2006a):

$$\frac{[A]}{k_0 - k} = \frac{1}{k_2 K_A} + \frac{[A]}{k_2} \quad (3)$$

where k_0 is the retention factor obtained in the absence of silanol suppressor and k is the retention factor obtained at a certain concentration of silanol suppressor [A]. It was apparent that higher the binding constants K_A were obtained for bromide and chloride derivatives than for tetrafluoroborate and sulfate whereas OMIM Br reduced silanol effect at the lowest concentration.

The ILs additives also significantly improve the asymmetry factor leading to higher column efficiencies especially for basic solutes (Martin-Calero et al., 2009; Tang et al., 2006; Xiao et al., 2004; He et al., 2003; Zhang et al., 2003; Jiang et al., 2008; Flieger & Czajkowska-Żelazko, 2011a,b,c; Flieger, 2009). It is obvious that dual nature of ionic liquids is responsible for observed effects. Whereas the ionic liquid cations undergo absorption onto the C-18 chain interacting with residual silanols, ILs anion depending on its position in the Hofmeister series acts as an ion-pairing or ion-exchanging agents.

Fig.11 shows the peak profile of pindolol analyzed by mobile phase without any ionic liquid additives and with addition of 10 mM BMIM Cl and 10 mM BMIM PF₆

Addition of BMIM Cl as mobile phase modifier provides a decrease of peak tailing and increase of efficiency in connection to retention decrease. Lack of ion-pairing properties of chloride anion and adsorption of ionic liquid cation on the surface provokes repulsion forces between protonated analytes and charged surface responsible for all advantageous effect. Addition of even 10 mM of BMIM PF₆ led to almost two-fold retention time increase as a result of ion-pair creation between chaotropic anion of added IL and protonated basic analyte involving worseness of efficiency and peak symmetry (Flieger & Czajkowska-Żelazko, 2011b).

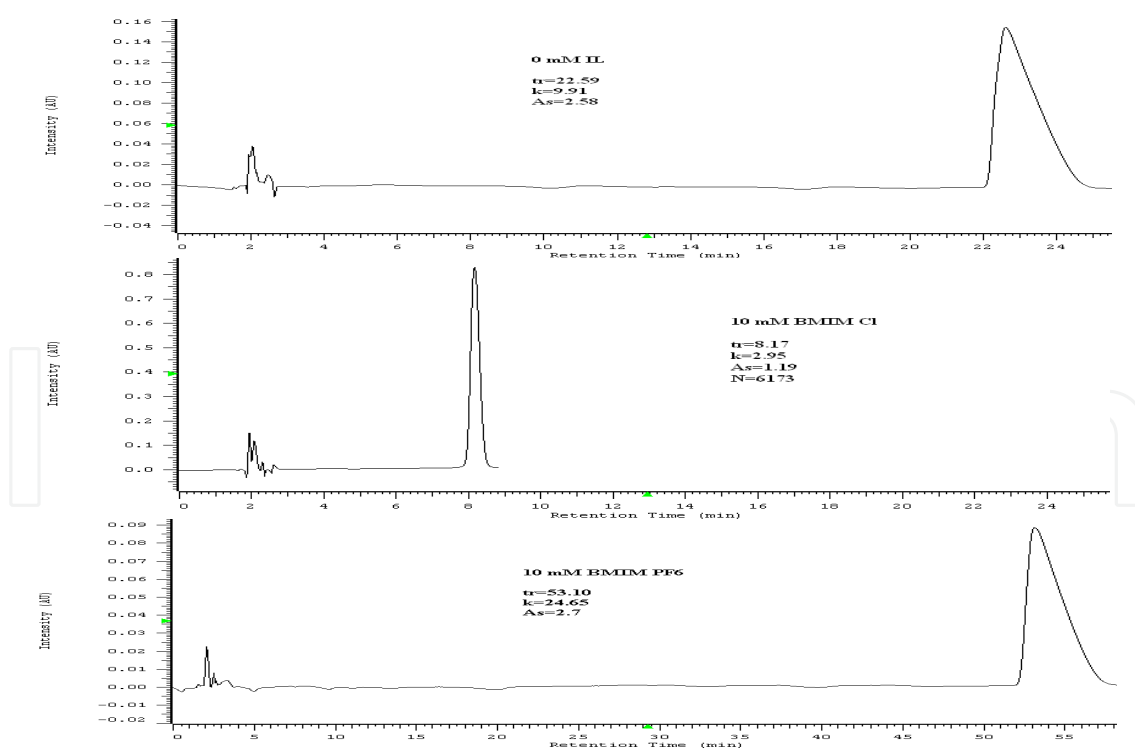


Fig. 11. Peak profile of pindolol obtained on Zorbax SB-Phenyl by the use of 10% ACN / 20 mM phosphate buffer pH = 2.9 mobile phase without any ionic liquid additives (0 mM ILs) and with addition of 10 mM BMIM Cl and 10 mM BMIM PF₆. (Flieger & Czajkowska-Żelazko, 2011b).

5.2 Stationary phases modification with ILs

Recently, ILs covalently bound to silica matrices have been described as stationary phases for liquid chromatography. Imidazolium-based ILs were applied for synthesis of anion exchange stationary phases by Qiu and coworkers (Qiu et al, 2006a,b) enabling to separate inorganic and organic anions. In turn Sun and coworkers using the same modifier synthesized stationary phase attaching IL (1-butyl-3-heptylimidazolium bromide) to silica matrix through an alkyl tether receiving column with properties similar to conventional phenyl stationary phase (Sun et al., 2005). Wang (Wang et al., 2006) modified the surface of 3 μm diameter silica particles with the following derivatives: 1-methyl-3-(trimethoxysilylpropyl) imidazolium bromide and 1-butyl-3-(trimethoxysilylpropyl) imidazolium bromide. It was confirmed by thermogravimetric analysis (TGA) and ^{13}C and ^{29}Si NMR spectroscopies that the ionic liquid moiety was predominantly attached to the silica surface through two siloxane bonds. The obtained columns were used for aromatic carboxylic acids separation under HPLC conditions (Fig.12). The major factors contributing to the retention of analytes on these types of columns appear to be ion-exchange process and the hydrophobic interactions.

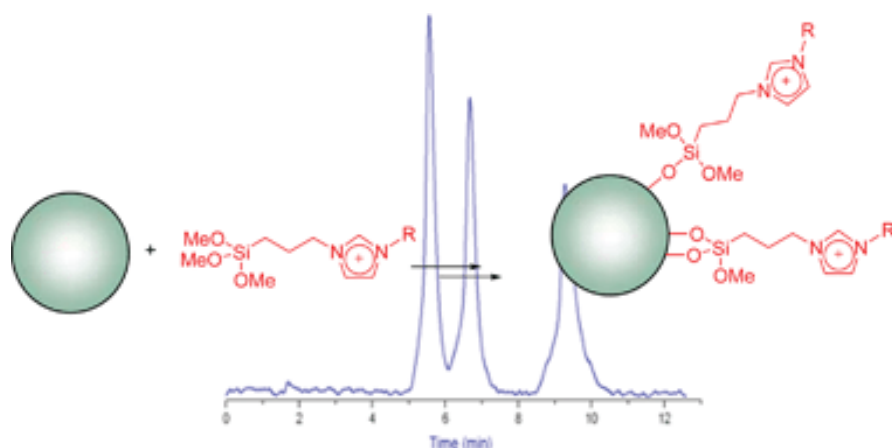


Fig. 12. Chromatogram of a mixture of acids eluted using 5 mM phosphate at pH 4.2 in an MPIM-modified silica particle packed column, using UV detection at a wavelength of 214nm. Flow rate is 200 mL/min. Peaks, from left to right: 3-(p-hydroxyphenyl) propionic acid (10mM), p-hydroxyphenylacetic acid (10mM), and benzoic acid (10mM) (Wang et al., 2006).

6. Application of ILs in capillary electrophoresis

Thanks to their good electrical conductivity, tunable viscosity and solubility, ILs could be applied as additives to electrolytes either aqueous or nonaqueous and as coating reagents of the capillary walls. The most popular approach appears to be application of ILs as running electrolytes. In this case the separation mechanism is complex and covers interactions of free IL ions with analytes in bulk solution and simultaneously dynamically coating the capillaries affecting an electroosmotic flow (EOF) and alleviating the wall adsorption usually occurring during separation of basic proteins by CE.

6.1 Modification the capillary wall with ILs

Ionic liquid (IL) could be covalently bonded onto the silica capillary surface affecting the electroosmotic flow due to the positive charges of the bonded ILs cation. Qin and Li (Qin &

Li 2002) determined sildenafil and its metabolite in human serum by capillary zone electrophoresis-mass spectrometry analysis using covalently bonded PMIM Cl to a silica capillary. Scheme representation of the IL coating procedure is presented on Fig.13. The adsorption of the analytes onto the bare capillary wall was eliminated by the IL coating and the drugs were baseline-separated within 14 min with detection limits of 14 and 17 ng/mL for sildenafil and metabolite respectively.

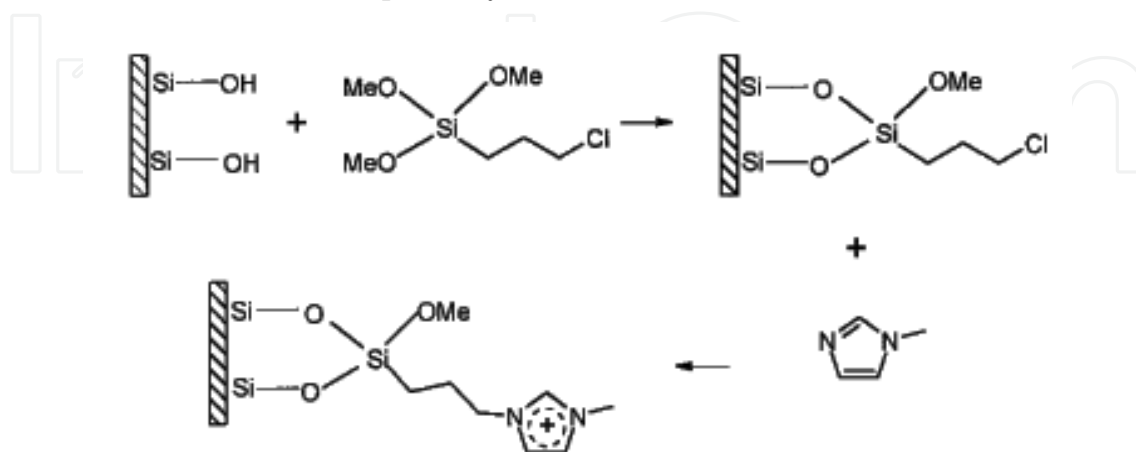


Fig. 13. Scheme representation of the IL coating procedure (Qin & Li, 2002).

6.2 ILs addition to the electrolyte

Use of ILs as background electrolytes in aqueous and non-aqueous electrophoresis is common practice. This approach has been applied for the separation of a variety of organic analytes (Table 3). The separation mechanism covers influence of either cation or anion of IL, however the resolving power is closely related to IL cation. The dynamic coating of the capillary wall by positively charged entire could reverse the electroosmotic flow and improve separations. The association between free ionic additives and ionized analytes could not be neglected. ILs were also used as additives in micellar electrokinetic chromatography (MEKC). Different ILs have been tested as modifiers in this chromatographic technique by Mwongela and coworkers (Mwongela et al., 2003) but only BMIM BF₄ could improve peak efficiency and resolution of tested mixtures of ketones, phenols and enantiomers of binaphthyl derivatives. ILs can be used not only as additives but also as surfactants. This approach requires appropriate length of the alkyl tail of the IL cation enabling micelles formation in aqueous media (Borissowa et al., 2008). The practical utilization of long-chain (C₁₂ and C₁₄) alkylimidazolium ionic liquids as a new type of surfactant in MEKC was used to separate neutral analytes-methylresorcinol isomers and benzene derivatives. Newly synthesized chiral ILs as surfactants in MEKC have been described in literature (Rizvi & Shamsi, 2006). Chiral separation of two acidic analytes, (±)-α-bromophenylacetic acid and (±)-2-(2-chlorophenoxy)propanoic acid (±)-(2-PPA) was achieved with both monomers and polymers of undecenoxycarbonyl-L-prolyrolidinol bromide and undecenoxycarbonyl-L-leucinol bromide at 25 mM surfactant concentration at pH 7.50. Authors claim that interaction of the acidic analytes with the cationic headgroup of chiral selectors contribute to observed the chiral recognition. Addition of chiral ILs could also improve enantioseparation using another chiral selector (Francois et al., 2007; Tran & Mejac, 2008). Two new chiral ILs (ethyl- and phenylcholine of bis(trifluoromethylsulfonyl) imide acting synergistically with classical cyclodextrins as chiral selectors, improved the enantioselectivity for 2-arylpropionic acids.

Ionic liquids added to the running electrolyte	Analyzed compounds	References
alkylammonium salts	Water-insoluble dyes	Vaher et al., 2000
tetramethylammonium tetrafluoroborate, 1-alkyl-3-methylimidazolium based ILs	Polyphenols in grape seed extracts	Yanes et al., 2000 Yanes et al., 2001
1-alkyl-3-methylimidazolium(BMIM OMIM)based ionic liquids with different anions: $[\text{PF}_6]^-$, $[\text{CH}_3\text{COO}]^-$, $[\text{CF}_3\text{COO}]^-$, $[(\text{CF}_3\text{SO}_2)_2\text{N}]^-$	Carboxylic acids and phenols: p-ethylphenol, p-cresol, phenol, hydroquinone, resorcinol, pyrocatechol, p-chloroglycinol, pyrogallol, 1,3-dihydroxynaphthalene.	Vaher et al., 2002 a,b
1-ethyl-3-methylimidazolium chloride (EMIM Cl) and the hexafluorophosphate salt (EMIM PF_6)	Chlorophenoxy and benzoic acid herbicides	Yu et al., 2005
1-ethyl-3-methylimidazolium tetrafluoroborate tetraethylammonium tetrafluoroborate	Monohalogenated phenols	Cabovska et al., 2003
dimethyldinonylammonium bromide	Carboxylates as copper complexes	Laamanen et al., 2005
1-alkyl-3-methylimidazolium based ionic liquids	Nicotinic, isonicotinic, picolinic acids	Marszałł et al., 2006 b
1-ethyl-3-methylimidazolium, 1-butyl-3-methylimidazolium, 1-pentyl-3-methylimidazolium, 1-heptyl-3-methylimidazolium tetrafluoroborates, 1-ethyl-3-methylimidazolium hexafluorophospha, 1-ethyl-3-methylimidazolium sulfate	Basic proteins: lysozyme, cytochrome c, trypsinogen, α -chymotrypsinogen A	Jiang et al., 2003
1-butyl-3-methylimidazolium tetrafluoroborate	Anthraquinone extracts	Qi et al., 2004
propane-1,3-bis(tripropylphosphonium)fluoride	Small organic anions	Krizek et al., 2009
1-ethyl-3-methylimidazolium, 1-butyl-3-methylimidazolium tetrafluoroborates, 1-butyl-3-methylimidazolium hexafluorophosphate	Flavone derivatives	Qi et al., 2006

Table 3. Use of ILs as background electrolytes.

7. Conclusion

The use of ILs in separation techniques has often been found to provide advantageous effects such as higher separation selectivity and efficiency of the systems. Recently developed ILs offer greater separation potential also for enantiomeric compounds. Special attention has been devoted to application of ILs as matrixes in matrix-assisted laser desorption ionization mass spectrometric analysis (MALDI-MS). Armstrong (Armstrong et al., 2001) was the first who noticed that low volatile ILs with good solvent properties could be suitable as liquid MALDI plates. To detect various types of molecules with good sensitivity and a wide detection range, new generation of ILs matrixes has been developed. It appeared that the cation of ILs used for this purpose should have $pK_a > 11$ and proton affinity higher than 930 kJ mol^{-1} (Crank & Armstrong, 2009). Quantification of large biomolecules such as proteins (Tholey et al., 2006) and synthetic polymers (Berthod et al., 2009) as well as low molecular weight compounds such as amino acids, sugars and vitamins (Zabet-Moghaddam et al., 2004) using ILs matrixes is a very promising approach of the last years. Additionally, considering environmental friendly properties of ILs, their separation applications will be further extensively explored.

8. References

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Applications of Ionic Liquids in Science and Technology

Edited by Prof. Scott Handy

ISBN 978-953-307-605-8

Hard cover, 516 pages

Publisher InTech

Published online 22, September, 2011

Published in print edition September, 2011

This volume, of a two volume set on ionic liquids, focuses on the applications of ionic liquids in a growing range of areas. Throughout the 1990s, it seemed that most of the attention in the area of ionic liquids applications was directed toward their use as solvents for organic and transition-metal-catalyzed reactions. Certainly, this interest continues on to the present date, but the most innovative uses of ionic liquids span a much more diverse field than just synthesis. Some of the main topics of coverage include the application of RTILs in various electronic applications (batteries, capacitors, and light-emitting materials), polymers (synthesis and functionalization), nanomaterials (synthesis and stabilization), and separations. More unusual applications can be noted in the fields of biomass utilization, spectroscopy, optics, lubricants, fuels, and refrigerants. It is hoped that the diversity of this volume will serve as an inspiration for even further advances in the use of RTILs.

How to reference

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Jolanta Flieger and Anna Czajkowska-Zelazko (2011). Ionic Liquids in Separation Techniques, Applications of Ionic Liquids in Science and Technology, Prof. Scott Handy (Ed.), ISBN: 978-953-307-605-8, InTech, Available from: <http://www.intechopen.com/books/applications-of-ionic-liquids-in-science-and-technology/ionic-liquids-in-separation-techniques>

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